

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
 US Department of Commerce
 United States Patent and Trademark
 Office, PCT
 2011 South Clark Place Room
 CP2/5C24
 Arlington, VA 22202
 ETATS-UNIS D'AMERIQUE
 in its capacity as elected Office

Date of mailing (day/month/year) 19 February 2001 (19.02.01)	
International application No. PCT/IL00/00363	Applicant's or agent's file reference Y/99-67 PCT
International filing date (day/month/year) 21 June 2000 (21.06.00)	Priority date (day/month/year) 21 June 1999 (21.06.99)
Applicant REVEL, Michel et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

02 January 2001 (02.01.01)

☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Claudio Borton Telephone No.: (41-22) 338.83.38
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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference Y/99-67 PCT	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/IL 00/ 00363	International filing date (day/month/year) 21/06/2000	(Earliest) Priority Date (day/month/year) 21/06/1999
Applicant YEDA RESEARCH AND DEVELOPMENT CO. LTD.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.



It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.



the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :



contained in the international application in written form.



filed together with the international application in computer readable form.



furnished subsequently to this Authority in written form.



furnished subsequently to this Authority in computer readable form.



the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.



the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

the text is approved as submitted by the applicant.



the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

the text is approved as submitted by the applicant.



the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

as suggested by the applicant.



because the applicant failed to suggest a figure.



because this figure better characterizes the invention.



None of the figures.



INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 00/00363

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/20 A61P25/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, CANCERLIT, AIDSLINE, LIFESCIENCES, CHEM ABS Data, SCISEARCH, BIOSIS, WPI Data, EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 02552 A (KOLLET ORIT ; LAPIDOT TSVEE (IL); CHEBATH JUDITH (IL); REVEL MICHEL) 21 January 1999 (1999-01-21) cited in the application page 1, line 5-13 page 126-9 claims 1,31,32,36,37 ---	1,5,9
A	RINGHEIM G E ET AL: "Enhancement of beta-amyloid precursor protein transcription and expression by the soluble interleukin - 6 receptor / interleukin - 6 complex." BRAIN RESEARCH. MOLECULAR BRAIN RESEARCH, (1998 MAR 30) 55 (1) 35-44. , XP000971298 the whole document / --- --/--	1-10



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

21 December 2000

Date of mailing of the international search report

11/01/2001

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Covone, M

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 00/00363

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	HAGGIAG S ET AL: "Induction of myelin gene expression in Schwann cell cultures by an interleukin-6 receptor-interleukin-6 chimera." FEBS LETTERS, (1999 AUG 27) 457 (2) 200-4. ' XP002156153 the whole document ----	1-10
P,X	DATABASE SCISEARCH 'Online! HAGGIAG S (REPRINT) ET AL: "Induction of myelin gene expression in cell cultures by an IL - 6 receptor - IL - 6 chimera." retrieved from STN Database accession no. 965500 XP002156155 abstract & NEUROSCIENCE LETTERS, (NOV 1999) SUPP. '54!, PP. S19-S19. PUBLISHER: ELSEVIER SCI IRELAND LTD, CUSTOMER RELATIONS MANAGER, BAY 15, SHANNON INDUSTRIAL ESTATE CO, CLARE, IRELAND. ISSN: 0304-3940., WEIZMANN INST SCI, DEPT MOL GENET, IL-76100 REHOVOT, ISRAEL ----	1-10
E	DATABASE WPI Section Ch, Week 200065 Derwent Publications Ltd., London, GB; Class B04, AN 2000-667404 XP002156156 & JP 2000 248000 A (TOSOH CORP), 12 September 2000 (2000-09-12) abstract -----	1,5,9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IL 00/00363

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9902552 A	21-01-1999	AU 8127198 A BR 9812096 A CN 1269835 T EP 0991661 A NO 20000086 A PL 338002 A ZA 9806145 A	08-02-1999 18-07-2000 11-10-2000 12-04-2000 08-03-2000 25-09-2000 07-04-1999
JP 2000248000 A	12-09-2000	NONE	

1. The first part of the document is a list of the names of the persons who have been named in the document. The names are listed in alphabetical order. The names are: [illegible]

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference Y/99-67 PCT	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) FOR FURTHER ACTION	
International application No. PCT/IL00/00363	International filing date (day/month/year) 21/06/2000	Priority date (day/month/year) 21/06/1999
International Patent Classification (IPC) or national classification and IPC A61K38/00		
Applicant YEDA RESEARCH AND DEVELOPMENT CO. LTD. et al.		
1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36. 2. This REPORT consists of a total of 6 sheets, including this cover sheet. <input type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of sheets.		
3. This report contains indications relating to the following items: <div style="margin-left: 40px;"> I <input checked="" type="checkbox"/> Basis of the report II <input type="checkbox"/> Priority III <input checked="" type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability IV <input type="checkbox"/> Lack of unity of invention V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement VI <input type="checkbox"/> Certain documents cited VII <input type="checkbox"/> Certain defects in the international application VIII <input type="checkbox"/> Certain observations on the international application </div>		
Date of submission of the demand 02/01/2001	Date of completion of this report 05.10.2001	
Name and mailing address of the international preliminary examining authority: <div style="display: flex; align-items: center;"> <div> European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465 </div> </div>	Authorized officer Perez, F Telephone No. +49 89 2399 7338	



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/IL00/00363

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-29 as originally filed

Claims, No.:

1-10 as originally filed

Drawings, sheets:

1/6-6/6 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/IL00/00363

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 9-10 (IA).

because:

☒ the said international application, or the said claims Nos. 9-10 (IA) relate to the following subject matter which does not require an international preliminary examination (*specify*):
see separate sheet

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)

Yes: Claims 2-4, 10

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/IL00/00363

	No:	Claims	1, 5-9
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-10
Industrial applicability (IA)	Yes:	Claims	1-8; for 9-10 see separate sheet
	No:	Claims	

2. Citations and explanations
see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/IL00/00363

Re Item III

Non-establishment of report with regard to novelty, inventive step and industrial applicability

Claims 9-10 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34.4(a)(i) PCT).

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

- D1:** WO 99 02552 A (KOLLET ORIT ;LAPIDOT TSVEE (IL); CHEBATH JUDITH (IL); REVEL MICHEL) 21 January 1999.
- D2:** RINGHEIM G E ET AL. BRAIN RESEARCH. MOLECULAR BRAIN RESEARCH, (1998 MAR 30) 55 (1) 35-44.
- D3:** HAGGIAG S (REPRINT) ET AL. NEUROSCIENCE LETTERS, (NOV 1999) SUPP. [54], PP. S19-S19.
- D4:** JP 2000 248000 A (TOSOH CORP), 12 September 2000.
- D5:** HAGGIAG S ET AL. FEBS LETTERS, (1999 AUG 27) 457 (2) 200-4.

Documents D3 and D4 are intermediate documents. The priority of the present application has been found to be valid. Therefore these documents were not considered as prior art.

Novelty (Articles 33.1 and 33.2 PCT)

Claim 1 relates to the use of a IL6RIL6 chimera for the manufacture of a medicament for the treatment and/or prevention of neurological diseases and disorders which is not novel with regard to claim 32 from D1.

Whereas the use of IL6RIL6 chimera for treating (or preparing a medicament against) neurological disease is known from D1, this document does not specifically disclose such use against traumatic nerve degeneration, demyelinating diseases of the CNS or PNS (

in particular MS) and/or neurodegenerative disease (in particular Alzheimer's disease, Parkinson's disease and ALS). Therefore **claims 2-4, 10** are novel.

Claims 5-8 relate to pharmaceutical composition comprising IL6RIL6 chimera which are not new with regard to claim 33 from D1. It should be noted that the indication of intended use of a substance is not considered distinctive characteristics which distinguishes it from the known form of this substance.

Claim 9 relates to a method of treating neurological disease using IL6RIL6 chimera which is not novel with regard to claim 31 from D1.

Inventive Step (Articles 33.1 and 33.3 PCT)

Traumatic nerve degeneration, demyelinating diseases of the CNS or PNS (in particular MS) and/or neurodegenerative disease (in particular Alzheimer's disease, Parkinson's disease and ALS) are not considered to be an inventive selection from the range of the neurologic diseases and disorders. Hence, no inventive step is acknowledged for **claims 2-4, 10**.

Industrial applicability (Articles 33.1 and 33.4 PCT)

Claims 1-8 cover compositions or their use for the manufacture of a medicament, and are therefore susceptible of industrial application.

For the assessment of the present **claims 9-10** on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 December 2000 (28.12.2000)

PCT

(10) International Publication Number
WO 00/78331 A2

(51) International Patent Classification⁷: **A61K 38/00**

Segrate, I-20090 Milano (IT). **BOSCHERT, Ursula**
[DE/CH]; Route d'Annecy 100 C, CH-1256 Troinex (CH).

(21) International Application Number: **PCT/IL00/00363**

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(22) International Filing Date: **21 June 2000 (21.06.2000)**

(25) Filing Language: **English**

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(26) Publication Language: **English**

(30) Priority Data:
130586 **21 June 1999 (21.06.1999)** **IL**

(71) Applicants (*for all designated States except US*): **YEDA RESEARCH AND DEVELOPMENT CO. LTD.** [IL/IL]; Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL). **APPLIED RESEARCH SYSTEMS ARS HOLDING N.V.** [NL/NL]; Pietermaai 15, Curacao (AN).

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **REVEL, Michel** [IL/IL]; Beit Brazil 5, Weizmann Institute of Science, 76100 Rehovot (IL). **CHEBATH, Judith** [IL/IL]; Rehov Miller 13, 76284 Rehovot (IL). **PIZZI, Marina** [IT/IT]; Via G. Peracchia, 8, Gussago, I-25064 Brescia (IT). **SPANO, PierFranco** [IT/IT]; Residenza Fontane 352,

Published:

— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **IL6RIL6 CHIMERA FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES**

(57) Abstract: The invention relates to the use of IL6RIL6 chimera in the treatment and prevention of neurological diseases and disorders.



WO 00/78331 A2

[illegible]

IL6RIL6 CHIMERA FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES

FIELD OF THE INVENTION

The present invention is generally in the field of neurological diseases and disorders. In particular it relates to neurodegenerative diseases, neuroprotection, nerve myelination and generation of cells which produce the myelin sheath. More particularly, the present invention provides for the use of IL6RIL6 chimeras for the manufacture of a medicament for treatment of neurological diseases or disorders, especially for neuroprotection and for the treatment of demyelinating diseases and the enhancement of nerve regeneration.

10 BACKGROUND OF THE INVENTION

Nerve myelination is an essential process in the formation and function of the central nervous system (CNS) and peripheral nervous system (PNS) compartments. The myelin sheath around axons is necessary for the proper conduction of electric impulses along nerves. Loss of myelin occurs in a number of diseases, among which are Multiple Sclerosis (MS) affecting the CNS, Guillain-Barre Syndrome, CIDP and others (see Abramsky and Ovadia, 1997; Trojaborg, 1998, Hartung et al, 1998). While of various etiologies, such as infectious pathogens or auto-immune attacks, demyelinating diseases all cause loss of neurological functions and may lead to paralysis and death. While present therapeutical agents reduce inflammatory attacks in MS and retards disease progression, there is a need to develop therapies that could lead to remyelination and recovery of neurological functions (Abramsky and Ovadia, 1997, Pohlau et al, 1998).

The synthesis of myelin is a function of specialized glial cells: the oligodendrocytes in the CNS and the myelinating Schwann cells in the PNS. These two cell types in their fully differentiated state may be called myelinating cells. Myelin is a lipid membrane structure containing a number of different proteins. Myelin basic proteins (MBP) represent the major components (30%) of CNS and also of PNS myelin proteins. Expression of the MBP and other genes encoding the various myelin proteins (e.g. P0, PMP-22, MAG in PNS, PLP, MOG in CNS), is turned on during the terminal differentiation of oligodendrocytes and



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myelinating Schwann cells. The origin of these cells is in the embryonal neural crest (Fraser, 1991) from which they migrate, and undergo a differentiation that proceeds in a number of steps. Schwann cell (SC) development appears to involve three main steps: 1) the generation of precursors (pSC) from migrating cells; 2) the proliferation and transition to embryonic SC (eSC) expressing the S100 protein; 3) the postnatal terminal differentiation of part of the eSC population into myelinating SC that express MBP and other myelin proteins (Kioussi and Gruss, 1996). The cells migrating from the neural crest give rise not only to pSC but also to sensory and sympathetic neurons, to smooth muscle cells and to cells which reach the skin and hair follicles and become pigmented melanocytes. The fate of the neural crest cells is affected by various inducing factors: differentiation to glial cells, to neurons and to muscles is promoted by Neuregulins such as glial growth factor (GGF), by BMP2/4 and by TGF- β respectively (Anderson, 1997). The differentiation to melanocytes may be promoted by growth factors such as bFGF or PDGF or SDF (Stocker et al, 1991; Anderson, 1997).

The ultimate differentiation of Schwann and oligodendrocyte progenitors into actively myelinating cells and myelination itself seems to depend on signals generated by the interaction between neuronal axons and the glial cells (Lemke and Chao, 1988; Trapp et al, 1988). When axon-Schwann cell contact is interrupted, as after nerve damage, the cells reverse to a non-myelinating state and expression of myelin protein genes is lost (Jessen and Mirsky, 1991). To be able to stimulate myelination or remyelination, after neural diseases or trauma, it would be extremely important to identify factors that are able to induce the synthesis of myelin.

Injury to CNS induced by acute insults including trauma, hypoxia and ischemia can affect both neurons and white matter. Although most attention has been paid to processes leading to neuronal death, increasing evidence suggests that damage to oligodendrocytes, which myelinate axons, is also a specific component of CNS injury. Thus oligodendrocyte pathology was demonstrated at very early phase after brain ischemia (3 hours) in rats, suggesting that these cells are even more vulnerable to excitotoxic events than neuronal cells (Pantoni et al. 1996). One potential candidate mediating cell death is the marked elevation of glutamate concentration that accompanies many acute CNS injuries (Lipton et al. 1994). Indeed, beside neurons even oligodendrocytes were found to express functional glutamate



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receptors belonging to the AMPA/kainate subtype. Moreover oligodendrocytes display high vulnerability to glutamate application (McDonald et al. 1998).

Neuregulins such as GGF, which act on embryonic Schwann cell precursors, are also survival, growth and maturation factors for postnatal oligodendrocytes and Schwann cells in damaged nerves, and GGF is one of the mitogenic factors provided by axonal contact (Topliko et al, 1996). Recombinant hGGF2 could enhance remyelination upon prolonged administration in a murine model for Multiple Sclerosis (Cannella et al, 1998) or in crushed peripheral nerve (Chen et al, 1998). Another cytokine that is induced in Schwann cells by axonal contact is the Ciliary neurotrophic factor CNTF (Lee et al, 1995). CNTF, as well as leukemia inhibitory factor (LIF), was shown to promote survival of oligodendrocytes from optic nerve cultured in vitro with bFGF or PDGF, and to increase the number of MBP expressing oligodendrocytes in these cultures (Mayer et al, 1994). However, when added to glial precursor cells, CNTF and LIF appear rather to favor astrocyte differentiation and induce expression of the astrocyte GFAP marker, while on oligodendrocytes it would have mainly a survival action with little effect on the level of MBP gene expression (Kahn and De Vellis, 1994, Bonni et al, 1997). Nevertheless, combinations of CNTF with brain-derived neurotrophic factor BDNF improve recovery of an injured peripheral sciatic nerve (Ho et al, 1998).

CNTF and LIF are cytokines acting through a common receptor system which comprises the LIF receptor (LIFR) and the gp130 chain, the latter being also part of the Interleukin-6 (IL-6) receptor complex (Ip et al, 1992). CNTF and LIF are, therefore, part of the IL-6 family of cytokines. In the case of CNTF and LIF, signal transduction operates through dimerization of LIFR with gp130, whereas in the case of IL-6 the signal is generated by the dimerization of two gp130 chains (Murakami et al, 1993). In order to bind gp130, IL-6 makes a complex with an IL-6 Receptor chain, which exists on certain cells as a gp80 transmembrane protein but whose soluble form can also function as an IL-6 agonist when provided from outside the cell (Taga et al, 1989, Novick et al, 1992). By fusing the entire coding regions of the cDNAs encoding the soluble IL-6 receptor (sIL-6R) and IL-6, a recombinant IL6RIL6 chimera can be produced in CHO cells (Chebath et al, 1997, WO99/02552). This IL6RIL6 chimera has enhanced IL-6-type biological activities and it

binds with a much higher efficiency to the gp130 chain in vitro than does the mixture of IL-6 with sIL-6R (Kollet et al, 1999).

A review of the effects of IL-6 on cells of the central and peripheral nervous system indicates that the cytokine may have protective effects on neuronal cells as well as participate in inflammatory neuro-degenerative processes (Gadient and Otten, 1997, Mendel et al, 1998). On glial cells, CNTF and LIF were much more active than IL-6 to stimulate astrocyte differentiation and there was no effect on myelin protein producing cells (Kahn and De Vellis, 1994). IL-6 was found to prevent glutamate-induced cell death in hippocampal (Yamada et al. 1994) as well as in striatal (Toulmond et al. 1992) neurons. The IL-6 mechanism of neuroprotection against toxicity elicited by NMDA, the selective agonist for NMDA subtype of glutamate receptors, is still unknown. In fact IL-6 was found to enhance the NMDA-mediated intracellular calcium elevation. In transgenic mice expressing higher levels of both IL-6 and soluble IL-6R (sIL6-R), an accelerated nerve regeneration was observed following injury of the hypoglossal nerve as shown by retrograde labeling of the hypoglossal nuclei in the brain (Hirota et al, 1996). In that work, the addition of IL-6 and sIL-6R to cultures of dorsal root ganglia (DRG) cells showed increased neurite extension in neurons, but no effect on myelinating cells was reported.

In the light of the data presented above, CNTF, LIF or a mixture of IL-6 and sIL-6R have not been shown to induce the terminal differentiation of glial cells into myelinating cells. However, as outlined above, stimulation of myelinating cells differentiation would be of great benefit for patients suffering from demyelinating or neurodegenerative diseases.

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SUMMARY OF THE INVENTION

It is the object of the present invention to provide means for the treatment and/or prevention of neurological diseases or disorders. In particular, it is an object of the present

invention to provide a means to stimulate or enhance the differentiation of progenitors or differentiated glial cells into myelinating cells. The basis of the invention is in the use of the recombinant IL6RIL6 chimera protein, which has a markedly higher affinity for gp130 than does the mixture of IL-6 and sIL-6R.

5 It is further an object of the present invention to provide a means to stimulate or enhance the myelination or remyelination of injured fibers as illustrated by the induced remyelination of axons following sciatic nerve axotomy *in vivo* further to the myelinating effects of the IL-6 chimera on the induction of myelin genes *in vitro*.

10 It is also an object of the present invention to use the IL6RIL6 chimera for increasing the number of Schwann cells developing in dorsal root ganglia (DRG) cultures. Furthermore, it is an object of the present invention to use the IL6RIL6 for induction of the differentiation of these cells to the point where they wrap around axons and produce myelin basic protein as illustrated in cocultures of Schwann cell lines with primary DRG.

15 It is another object of the present invention to use the IL6RIL6 chimera for induction the transcription of the myelin basic protein (MBP) genes in a system of transdifferentiation in which cells with melanocytic phenotype are converted into a Schwann, myelinating phenotype, as illustrated by the effect of IL6RIL6 on a murine melanoma.

20 It is another object of the present invention to use the IL6RIL6 chimera as a neuroprotective agent and for prevention of neuronal cell death in the hippocampus, a region involved in memory encoding and one that exhibits early degeneration in Alzheimer's disease and ischemia.

25 It is another object of the present invention to use the IL6RIL6 chimera as a protective agent against the neurotoxicity process triggered by excitatory amino acids as illustrated by the protection from glutamate-induced neurotoxicity provided by the IL-6 chimera in primary cultures of new born rat cerebella neurons.

A molecular mechanism is proposed by which the IL6RIL6 induces and represses specific transcription factors that bring about the induction of the MBP gene differentiation into myelinating phenotype.

30 Thus, the present invention provides the use of IL6RIL6 chimera for the manufacture of a medicament for the treatment and/or prevention of neurological diseases and disorders.

In particular, the present invention provides the use of IL6RIL6 chimera for the manufacture of a medicament for treating traumatic nerve degeneration, demyelinating diseases of the CNS or PNS and/or neurodegenerative diseases.

5 More particularly, the invention provides for the use of IL6RIL6 chimeras in the treatment of multiple sclerosis (MS), Alzheimer's disease, Parkinson's disease or ALS.

The invention also provides pharmaceutical compositions comprising IL6RIL6 chimera, optionally together with one or more pharmaceutically acceptable excipients, for the treatment and/or prevention of neurological disease and disorders. In particular the pharmaceutical composition is for treating traumatic nerve degeneration, demyelinating
10 diseases of the CNS or PNS and/or neurodegenerative diseases.

A preferred use for the pharmaceutical compositions of the present invention is the treatment of MS, Alzheimer's disease, Parkinson's disease or ALS.

BRIEF DESCRIPTION OF THE DRAWINGS

15 Fig. 1 shows the increase in MBP RNA in cultures of Schwann cells treated by IL6RIL6 chimera or by forskolin (FSK) or left untreated (NT).

Fig. 2 shows the proliferation of undifferentiated oli-neu cells measured after 24, 48 and 72 hours after treatment with different amounts of IL6R/IL6 chimera.

20 Fig. 3 shows that IL6RIL6 strongly induces MBP mRNAs (A) and decreases Pax-3 mRNA (B) over time in the F10.9 cells. NT designate non treated cells.

Fig. 4 shows a comparison of neuroprotection by IL-6 alone and the IL-6 chimera on NMDA mediated neurotoxicity in hippocampal organotypic slices.

Fig. 5 shows the prolonged neuroprotective effect of the IL-6 chimera vs. the effect of IL-6 alone in hippocampal organotypic slices.

25 Fig. 6 shows the effect of IL6R/IL6 chimera on the survival of NGF-deprived neurons after 24 hours of treatment as measured by the MTT assay.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the invention it has been found that addition of IL6IL6R recombinant protein to cultures of dorsal root ganglia cells or of melanoma cells stimulates the differentiation of those cells into myelinating cells. It was also found that the addition of the IL6IL6R recombinant protein to cocultures of neurons and Schwann cells induces the latter to form a regular sheath around the axons. The invention therefore relates to the use of the IL6RIL6 chimera for the manufacture of a medicament to generate myelinating cells or to stimulate, enhance or accelerate the generation of myelinating cells.

The IL-6 chimera further induces *in vivo* remyelination of transected fibers following sciatic nerve axotomy in rats. The invention therefore further relates to the use of IL6RIL6 chimera for manufacture of a medicament to induce, enhance or accelerate remyelination, in particular after nerve damage or trauma or axonal injury.

The addition of IL6IL6R recombinant protein to organotypic cultures was further found to induce a prolonged protective effect from neurotoxic agents. The invention therefore also relates to the use of IL6RIL6 chimera for manufacture of a medicament to induce, enhance, prolong or accelerate neuroprotection, in particular from neurotoxic agents, and to inhibit, reduce or decelerate neuronal death, which may be due to apoptosis, for example.

The present invention concerns the use of the "IL6RIL6 chimera" (also called "IL6RIL6" or IL-6 chimera), which is a recombinant glycoprotein obtained fusing the entire coding sequence of the naturally-occurring soluble IL-6 Receptor δ -Val to the entire coding sequence of mature naturally-occurring IL-6, both from human origin. The IL6RIL6 chimera may be produced in any adequate eukaryotic cells, such as yeast cells, insect cells, and the like. It is preferably produced in mammalian cells, most preferably in genetically engineered CHO cells as described in WO 99/02552. Whilst the protein from human origin is preferred, it will be appreciated by the person skilled in the art that a similar fusion protein of any other origin may be used according to the invention, as long as it retains the biological activity described herein.

More particularly, the present invention concerns the use of IL6RIL6 chimera to stimulate the differentiation of progenitor or differentiated glial cells into myelinating cells.

As demonstrated herein the IL6RIL6-induced myelinating cells differentiation process involves both, activation of genes required for the formation of the myelin sheath around neuronal axons, as well as repression of a gene required for the maintenance of non myelinating phenotypes.

5 In accordance with the present invention it has been observed that the addition of IL6RIL6 chimera to cultures of embryonic dorsal root ganglia (eDRG) cells, isolated from mice embryos at days 14-15 of gestation, has a profound effect on the development of the Schwann cell precursors present in the DRG. After 2-5 days in culture, there is a marked increase in the number of the embryonic Schwann cells, a marked phenotypic change in
10 these cells, which start to wrap their membrane around the DRG axons, and an induction of MBP. In accordance with the present invention it has been further observed that in a co-culture of Schwann cells with neurons the IL-6 chimera can induce a significant increase in the binding of Schwann cells along the non-myelinated axons within 5 hours. The Schwann cells, which were labeled with fluorogold, elongate and after a few days their
15 fluorescent cytoplasm could be seen to form a regular sheath around the axons. Without the chimera, or with NGF, the Schwann cells bind much less and do not form a sheath.

Therefore, the invention further relates to the use of IL6RIL6 chimera for the manufacture of a medicament to induce Schwann cell proliferation and/or differentiation, as well as to the myelination by Schwann cells in the peripheral neural system.

20 In accordance with the present invention it has further been shown that the IL-6 chimera can induce remyelination of peripheral nerves *in vivo*. Following sciatic nerve axotomy in rats and juxtaposition of proximal and distal stumps the IL-6 chimera could induce the regeneration of the peripheral nerves and the remyelination of the transected fibers *in vivo*. In the presence of the IL-6 chimera a 4 fold increase in the number and
25 thickness of myelinated fibers was found and an increase in the remyelination of the more distant fibers. Therefore, the invention further relates to the use of IL6RIL6 chimera for the manufacture of a medicament to induce remyelination in the peripheral nervous system.

Furthermore it has also been found according to the present invention that the IL-6 chimera can induce the expression of the genes encoding myelin protein components such as
30 MBP, PLP and P0 genes in myelinating cells of the peripheral nervous system such as Schwann cells, and in cells of the central nervous system such as oligodendrocytes.

Therefore, the invention further relates to the use of IL6RIL6 chimera for the manufacture of a medicament to induce myelination and/or remyelination by oligodendrocytes in the central nervous system.

It has also been found according to the present invention that the addition of IL6RIL6 chimera to cultures of the B16/F10.9 murine melanoma cell line, induces the expression of the MBP gene within 6-12 hours. Other genes, which encode proteins of the myelin, such as the CNPase gene, are induced whereas expression of genes which are involved in melanogenesis (formation of melanin pigments) such as tyrosinase, are strongly repressed. The F10.9 cells treated by IL6RIL6 also undergo a marked morphological change, and acquire a Schwann-like phenotype. The phenotypic changes and the induction of specific myelin genes support the hypothesis that IL6RIL6 causes a transdifferentiation of the cells from a melanocytic to myelinating state. Since in the embryo, cells migrating from the neural crest can give rise to either melanocytes or myelinating Schwann cells and oligodendrocytes, it is suggested that IL6RIL6 can influence the fate of the cells and promote the formation of myelinating cells. Therefore, the invention also relates to the use of IL6RIL6 chimera for the manufacture of a medicament to induce, promote, enhance or accelerate the formation of myelinating cells in the peripheral and in the central nervous system and/or to induce transdifferentiation of melanocytic cells into myelinating cells.

Moreover, it is shown in accordance with the present invention that IL6RIL6 acts by down-regulating the homeobox gene Pax-3, a gene expressed in embryonic neural crest cells before they differentiate into myelinating Schwann cells (Kioussi and Gruss, 1996). Pax-3 is known to repress MBP gene. Therefore, Pax-3 repression appears to be a key event in the final maturation of the myelinating cells. Hence IL6RIL6 acts on a key differentiation switch (i.e. pax-3 repression).

Pax-3 is a transactivator of the microphthalmia associated transcription factor MITF, which in turn induces and maintains the expression of the tyrosinase and other genes responsible for the melanocytic phenotype. The discovery of the rapid repression of Pax-3 by IL6RIL6 can therefore explain the molecular events which promote the myelinating activity of the neural crest derived cells. After nerve injury, myelinated axons undergo demyelination as part of the Wallerian degeneration. During that process, Schwann cells turn down the expression of MBP gene and other related myelin protein genes. Concomitantly there is an

upregulation of Pax-3 and GFAP that denotes a reversion from myelinating SC to non-myelinating and proliferating SC (Kioussi and Gruss, 1996). In accordance with the present invention, the IL6RIL6 chimera appears to be a potent cytokine to revert the process of Wallerian nerve degeneration by repressing Pax-3 and inducing the SC to resume their myelinating activities. The same considerations would apply to brain demyelinating diseases since, like in traumas, the neurodegeneration in these diseases is spurred by a demyelination process driven by macrophages and other inflammatory cells. The invention therefore also relates to the use of IL6RIL6 chimera for the manufacture of a medicament for treatment of nerve damage and/or traumatic nerve degeneration and/or axonal injury.

IL6RIL6 can be injected to mice in which an autoimmune demyelination has been induced by immunization with MBP as a model system for chronic relapsing multiple sclerosis (Cannella et al, 1998). The capacity of IL6RIL6 to induce myelin protein genes and differentiation of myelinating glial cells can be observed in vivo, using this pharmaceutical paradigm. The invention therefore further relates to the use of IL6RIL6 chimera for the manufacture of a medicament for treatment and/or prevention of autoimmune demyelinating diseases, in particular to the treatment and/or prevention of Multiple Sclerosis.

The IL-6 chimera has been also shown to have neuroprotective effects, preventing loss of neuronal cell viability induced by excitotoxic agents in regions involved in memory encoding and exhibiting early degeneration in Alzheimer's disease and ischemia. The IL-6 chimera was found to protect neurons from glutamate induced neurotoxicity. Therefore, the invention relates to the manufacture of a medicament for the protection of neural cells against cell death, in particular due to the effects of neurotoxic agents, and to the treatment and/or prevention of neurodegenerative diseases like Alzheimer's disease, Parkinson's disease or ALS, for example.

Further neurological diseases that may be treated with the IL6RIL6 chimera are strokes, movement disorders, epilepsy, pain and the like.

The definition of "pharmaceutically acceptable" is meant to encompass any carrier, which does not interfere with effectiveness of the biological activity of the active ingredient and that is not toxic to the host to which it is administered. For example, for parenteral administration, IL6RIL6 chimera may be formulated in a unit dosage form for injection in vehicles such as saline, dextrose solution, serum albumin and Ringer's solution.

The IL6RIL6 chimera can be administered to a patient in need of administration thereof in a variety of ways. The routes of administration include intradermal, transdermal (e.g. in slow release formulations), intramuscular, intraperitoneal, intravenous, subcutaneous, oral, epidural, topical, and intranasal routes. Any other therapeutically efficacious route of administration can be used, for example absorption through epithelial or endothelial tissues or by gene therapy wherein a DNA molecule encoding the IL6RIL6 chimera is administered to the patient (e.g. via a vector) which causes the IL6RIL6 chimera to be expressed and secreted *in vivo*. In addition the IL6RIL6 chimera can be administered together with other components of biologically active agents such as pharmaceutically acceptable surfactants, excipients, carriers, diluents and vehicles.

For parenteral (e.g. intravenous, subcutaneous, intramuscular) administration, IL6RIL6 chimera can be formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle (e.g. water, saline, dextrose solution) and additives that maintain isotonicity (e.g. mannitol) or chemical stability (e.g. preservatives and buffers). The formulation is sterilized by commonly used techniques.

An "effective amount" refers to an amount of the active ingredients that is sufficient to affect the course and the severity of the diseases described above, leading to the reduction or remission of such pathology. The effective amount will depend on the route of administration and the condition of the patient.

The dosage administered, as single or multiple doses, to an individual will vary depending upon a variety of factor, including IL6RIL6 chimera pharmacokinetic properties, the route of administration, patient conditions and characteristics (sex, age, body weight, health, size), extent of symptoms, concurrent treatments, frequency of treatment and the effect desired. Adjustment and manipulation of established dosage ranges are well within the ability of those skilled, as well as *in vitro* and *in vivo* methods of determining the remyelination of the nerves.

While the invention will be described in conjunction with specific embodiment thereof, it will be understood that it is capable of further modifications. This application is intended to cover any variations, uses or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as

come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth as follows in the scope of the appended claims.

All references cited herein, including journals articles or abstracts, published or unpublished patent applications, issue or foreign patents, or any other references, are entirely incorporated by references herein, including all data, tables, figures, and text presented in the cited references. Additionally, the entire contents of the references cited within the references cited herein are also entirely incorporated by references.

The present invention will now be described in more detail in the following non-limiting Examples and the accompanying drawings.

EXAMPLES

Example 1: Effect of IL6RIL6 on myelination and remyelination *in vitro*

In the spinal cord, the dorsal root comprises essentially sensory neurons, which form synapses in the dorsal root ganglia (DRG). During mouse embryogenesis (at day e14-e15), DRGs are a convenient source of neurons and of embryonic Schwann cells which have not yet differentiated into myelinating SC. Procedures to obtain explants of DRGs for in vitro cultures are described by Li (1998). The cultures were performed on glass coverslips placed in the wells of Costar plates, in medium F12/DMEM (Gibco). The coverslips were coated either with collagen or with poly-D-lysine, with essentially similar results. Cultures were either done in medium without growth factor or cytokine additions or in medium supplemented with nerve growth factor (NGF, 40 ng/ml), or in medium supplemented with IL6RIL6 chimeric recombinant proteins (3 µg/ml). The cultures were examined daily by light microscopy with an Olympus inverted microscope linked to a video-camera imaging system (Leica LIDA system). Part of the coverslips were fixed in paraformaldehyde and MBP proteins were labeled with monoclonal primary antibodies to myelin basic proteins and fluorescein-conjugated secondary antibody. Neuronal cell bodies and axons were stained with antibodies to neurofilament protein. Some of the coverslips were examined by scanning electron microscopy (EM).

After 2 to 5 days, the DRG explants cultured without addition showed that the cells growing out of the explant were either polygonal or oval-shaped. However, when NGF was added, the oval shaped cells developed long axonal projections, which formed a thin network stained by antibodies to neurofilaments. Some of the axons were long and bifurcated, but no Schwann cells were observed along the axons. In contrast, cultures with IL6RIL6 showed not only neuronal cells with axons stained for neurofilament, but also Schwann cells appearing as flat cells that had long bipolar extensions with end ramifications. These extensions were not stained for neurofilament proteins. By scanning EM, these Schwann cells were clearly observed along the axonal projections with membrane rufflings starting to wrap around the axon.

Staining with anti-MBP showed positively stained Schwann cells in the IL6RIL6-treated cultures, in particular in arrays of cells which were aligned one after the other. On the other hand, little MBP-specific fluorescence was seen in the NGF-treated cultures without IL6RIL6.

Similar results were observed in DRG cultures from e15 rat embryos.

Schwann cells derived from mouse sciatic nerve were also cultured in vitro with IL6RIL6 1.4 µg/ml and the level of MBP RNA transcript was measured. In comparison, the same cultures were treated with forskolin 20 µM, a chemical which artificially increases the cyclic AMP levels in the cells and is known to induce MBP (Lemke and Chao, 1988). The results showed that IL6RIL6 was as efficient as forskolin to induce MBP gene expression and more efficient to maintain MBP RNA levels after 3 days of culture (Fig. 1).

In a study in 18-day embryo dorsal root ganglion (DRG) cells the IL-6 chimera was found to induce MBP and P0 mRNA expression within 3 days, whereas Pax-3 mRNA was strongly inhibited in the same cell system. A dose-dependent curve of the IL-6 chimera effect on the P0 and MBP gene expression shows maximal effect of the IL-6 chimera at 500 ng/ml. The IL-6 chimera appears, therefore, as an important inducer of myelin gene expression in normal neuroglial cells.

Schwann cell-lines derived from rat sciatic nerve or from rat Dorsal root Ganglion (DRG) cells were generated for further studying the effects of the IL-6 chimera.

The Schwann cell-line derived from rat sciatic nerve was found to respond to the IL-6 chimera, as determined by RT-PCR and Northern blot analysis, by a 2-3 fold P0 gene induction, whose protein product forms about 50% of peripheral nerve myelin.

5 In order to study the transcriptional activation of the myelin gene components by the IL-6 chimera the MBP promotor was introduced into an expression vector upstream to the luciferase reporter gene and tested in the rat sciatic nerve Schwann cell line. Up to 7-fold induction of the transcriptional activity from the MBP promotor was observed in response to the IL-6 chimera in these cells in the absence of forskolin. In an additional reporter gene assay the IL-6 chimera was found to induce a 2.5 fold induction in the transcription from the
10 promotor of the P0 gene in these cells.

The above results suggest that the IL-6 chimera has a direct effect on myelin gene transcription in committed Schwann cells.

An additional Schwann cell-line was isolated from the rat Dorsal Root Ganglion (DRG) and named the CH-cell line. This cell line was found to have a slow growth rate and
15 to be dependent for growth on the IL-6 chimera (140ng/ml). Interestingly, the CH-cells had a stellar morphology resembling that of oligodendrocytes. The cells appeared to be functional with respect to induction of myelination by the IL-6 chimera. The cells expressed P0 and MBP and died when grown in serum containing medium in the absence of the chimera.

20 **Example 2: Inhibition of oligodendrocyte proliferation *in vitro***

The effects of the IL-6 chimera were studied on cells of the central nervous system. It is established that oligodendrocyte differentiation is associated with the inhibition of their proliferation, thus promoting sheath formation and myelination by oligodendrocytes.

Therefore, the capacity of the IL6R/IL6 chimera to inhibit oligodendrocyte
25 proliferation was tested *in vitro*. A murine primary oligodendrocyte (oligodendroglial) cell line immortalized with the t-neu oncogene ("oli-neu" cell line) was used in this experiment. The establishment and properties of the oli-neu cell line as well as the culturing conditions are described in Jung et al. (1995).

The proliferation of undifferentiated oli-neu cells was measured after 1, 2 and 3 days in response to different amounts of IL6R/IL6 chimera (1µg/ml, 500ng/ml, 250ng/ml, 125ng/ml and 0 ng/ml (control)). The growth rate was quantified by measuring the cellular metabolic activity with a fluorometric/colorimetric growth indicator, Alamar Blue. This agent contains an oxidation-reduction indicator that shows both fluorescence and changes its color in response to chemical reduction of growth medium resulting from cell growth. The agent and assay used are described in Ahmed et al. (1994) and the US patent 5,501,959.

The results are shown in Fig. 2. Addition of IL6R/IL6 chimera to the culture medium of oligodendrocytes led to a pronounced reduction of growth already after 48 hours. After 72 hours, the growth was reduced to 40 to 50% as compared to the control. Interestingly, the highest amount of IL6R/IL6 chimera (1 µg) was less effective than the lower amounts of 500 to 125 ng/ml.

This experiment shows that the IL6R/IL6 chimera strongly inhibits proliferation of oligodendrocytes *in vitro*, indicating that it is a promoter of oligodendrocyte differentiation. Since oligodendrocytes are the glia cells producing myelin sheaths in the CNS, the IL6R/IL6 chimera may be an agent actively promoting remyelination *in vivo*.

Example 3: Impact of IL6R/IL6 chimera on oligodendrocyte morphology

In order to study the effect of IL6 Chimera on the morphology of oligodendroglia, oli-neu cells (see Example 2) were cultured for 3 days in the presence of 1 µg/ml of IL6R/IL6 chimera.

Dibutyl cAMP (dbcAMP) is an agent known to induce differentiation in oligodendrocytes (see, e.g. Jung et al. (1995)). In order to investigate, which morphological effect the IL6R/IL6 chimera adds to the effect of dbcAMP, oli-neu cells were pre-treated for 3 days with dbcAMP before addition of IL6R/IL6 chimera plus dbcAMP. In parallel, cells were treated with the IL6R/IL6 chimera without pre-treatment.

Changes in morphology were assessed with phase contrast microscopy or by immunohistochemistry staining for oligodendrocyte markers CNPase (2',3'-cyclic-nucleoside-3'-phosphodiesterase) and GalC (sialogangliosides and sulfatides) and using the antibody A2B5, specific for the myelin lipid galactocerebroside.

The addition of IL6R/IL6 chimera clearly induced morphological changes in the oli-neu cells. Cell bodies assembled in rows and their processes became more elongated. The cells themselves were elongated and seemed to be fused, indicating an advanced state of differentiation.

5 The expression of oligodendrocyte markers was shown by immunohistochemistry. Cells were positive for the oligodendrocyte specific markers A2B5-antigen and CNPase, but were negative for the astroglial marker GFAP, thus showing that indeed, the oligodendrocyte phenotype was maintained.

10 DbcAMP pretreatment and combined treatment with IL6R/IL6 chimera and dbcAMP increased the number of sheath forming oligodendrocytes after 3 days. Besides elongated cells seen with IL6R/IL6 chimera alone, the morphology of the cells looked even more differentiated. Cells having a sheet-like shape could be observed, indicating an advanced differentiation state.

15 In conclusion, treatment with the IL6R/IL6 chimera resulted in a morphological change of oligodendrocytes *in vitro*, thus further supporting its role as an inducer of oligodendroglial differentiation and myelination.

Example 4: IL-6 chimera effects on neuronal Schwann cell interaction

20 By culturing murine DRG in the presence of DNA synthesis inhibitors arabinoseC (AraC) and fluorodeoxyuridine (FudR), neuronal cells can develop axons while glial cell proliferation is inhibited. These DRG cultures can be used as a source of neuronal cells with non-myelinated axons. Schwann cells are exogenously added to these cultures to study the interaction between neurons and Schwann cells.

25 Rat sciatic nerve Schwann cell lines cells were labeled with fluorogold, a label that penetrates the cell membrane lipid bi-layer and is maintained for an extensive period of time without affecting cell function. Co-culture of the neuronal cells with these Schwann cells in the presence of the IL-6 chimera leads to a significant increase in the binding of the Schwann cells along the axons cells within 5 hours. The cells elongate and after a few days their fluorescent cytoplasm can be seen to form a regular sheath around the axons. Without the
30 chimera. or with NGF, the Schwann cells bind much less and do not form a sheath. These

results show that the IL-6 chimera has a very rapid effect on the interaction of glial cells with neurons, suggesting that adhesion molecules are induced or activated. This system allows to study the various steps in the myelination process which are affected by the IL-6 chimera.

5 **Example 5: Effect of IL6R/IL6 chimera on myelination in mixed cultures of neurons and oligodendrocytes**

10 In order to further investigate the myelination inducing activity of IL6R/IL6 chimera, dissociated cultures from murine E15 (embryonal day 15) cerebral hemispheres were prepared. The preparation and culture conditions are described by Lubetzki et al. (1993). The primary cells were maintained in culture for 8 days before addition of IL6R/IL6 (1.5ug/ml) for 11 days.

15 MBP (myelin basic protein) and the oligodendrocyte specific protein PLP (ProteoLipid Protein) are markers for mature and/or myelinating oligodendrocytes and are major constituents of CNS myelin. They can therefore be used as markers for assessing active myelination taking place in the cell culture.

Under the conditions of the culture, at day 4, there is no myelination. Therefore, day 4 mRNA was used as a calibrator control in this experiment. At day 19, under the culture conditions, myelination in the cell culture is observed.

20 Thus at days 4 and 19, mRNA was extracted from treated and non-treated wells, in order to quantify MBP and PLP mRNA.

The mRNAs for MBP, PLP and GAPDH, which served as an internal control RNA, were measured by real-time quantitative RT-PCR (Medhurst et al. (2000) using a PE Applied Biosystems Prism model 7700 sequence detection instrument.

25 The results are summarized in Table I below. Two independent experiments were carried out. The mRNA expression is indicated as fold expression of the control RNA at day 4. In both experiments, the IL6R/IL6 chimera clearly enhanced the expression of MBP and PLP by twofold, again supporting the role of IL6R/IL6 chimera as an inducer or enhancer of myelination.

TABLE I: MBP and PLP expression in primary cortical cultures

	<u>Control day 4</u>	<u>Control day 19</u>	<u>IL6R/IL6 chimera day 19</u>
<u>Experiment I</u>			
MBP	1	72	181
PLP	1	1265	2157
<u>Experiment II</u>			
MBP	1	187	2157
PLP	1	1884	4198

In conclusion, examples 1 to 5 show that the IL6R/IL6 chimera has an anti-proliferative, differentiation and myelination inducing activity. This activity of IL6R/IL6 chimera strongly suggests a beneficial effect of IL6R/IL6 chimera in peripheral and central nervous system remyelination. This effect can be exploited in all disorders based on myelination defects, demyelination or insufficient myelination of the CNS or PNS neurons. The IL6R/IL6 chimera may therefore be an effective agent to treat re- and/or demyelination disorders like Multiple Sclerosis, for example.

Example 6: The effect of the IL-6 chimera on peripheral nerve regeneration *in vivo*

Following sciatic nerve axotomy in rats and juxtaposition of proximal and distal stumps, peripheral nerves can be induced to regenerate and transected fibers can be induced to remyelinate *in vivo* (Sahenk et al. 1994). The effect of the IL-6 chimera on remyelination of peripheral nerves following axotomy was examined. The rats (7 animals) were given sIL-6/IL-6 chimera intraperitoneally every second day at a dose of 100 mcg/Kg for 12 days beginning on the day of surgery. Control animals (9 rats) were injected with PBS. After 12 days, sections of the regenerating nerve 2.5 and 5 mm below axotomy were analysed by transmission electron microscopy and the number of positive fibers was evaluated.

In the presence of the IL-6 chimera a 2.5-fold increase in the number of myelinated fibers was found at a 2.5-mm distance below axotomy compared to PBS treated controls.

The chimera was further found to increase the remyelination of the more distant fibers inducing a 5.2 fold increase in the number of myelinated fibers at 5mm distal to the section. This is important since remyelination decreases as the distance from the section increases. CNTF effects have been observed at 0.5 mm from the section, but with the IL-6 chimera the effect seems much more extended and greater at 5mm than at 2.5 mm. Also the thickness of myelinated fibers increased over 2 fold after treatment with the chimera. Overall the IL-6 chimera appears to induce remyelination of about 10% of fibers as compared to intact non-axotomised rats.

Example 7: Induction of MPB gene by IL6RIL6 in cultures of melanoma B16-F10.9 cells

The embryonic origin of skin melanocytes, which produce the melanin pigments, and of Schwann cells is from common precursor cells migrating out of the neural crest in e8 mouse embryos. Melanomas are malignant tumors developing in the skin from melanocytes, and are therefore also derived from neural crest ancestors. The B16 cell line is derived from a spontaneous melanoma of Balb/c mice, and the F10.9 clone was isolated from B16 for its highly malignant metastatic phenotype. The F10.9 cells, as other B16 cells, produce black eumelanin pigment and are rich in tyrosinase, the first enzyme of the melanogenic pathway (Bertolotto et al, 1996).

F10.9 cells were seeded in 96-well microplates at 30,000 cells/well and cultured for 3 days in DMEM medium with 10% FCS, without or with IL6RIL6 at concentrations of 0.3-1 µg/ ml. Total cell RNA was extracted and analysed by Northern blots with cDNA probes for MBP. The MBP mRNAs were induced very strongly at 48 by IL6RIL6 in the F10.9 cells (Fig. 3A). A time course study showed that the increase in MBP RNA started at 12 hours following the addition of the IL6RIL6 chimera to the cell cultures.

The IL6RIL6 chimera induces not only MBP gene expression but also the cyclic 2'3' AMP phosphodiesterase or CNPase, which is another component of the myelin and a marker for differentiated Schwann cells. The cells also developed prolonged extensions at opposite poles of the cell body, and aligned in long arrays as typical of Schwann cells in cultures.

Surprisingly, IL6RIL6 switched the phenotype of the F10.9 cells from melanin producing cells to myelin producing Schwann cells. The tyrosinase enzymatic activity and the production of melanin were completely lost at 48 hours after addition of the IL6RIL6 chimera to the cells.

MITF is a transcription factor that activates the tyrosinase gene (Bertolotto et al, 1996). The treatment of F10.9 cells by IL6RIL6 strongly repressed the MITF gene expression. MITF itself is transactivated by the homeotic transcription factor Pax-3 (Watanabe et al, 1998) and measurements of Pax-3 mRNA in the IL6RIL6 treated F10.9 cells showed that Pax-3 expression decreases starting from 6 hours and up to 48 hours (Fig. 3B). Pax-3 is known to repress the MBP gene (Kioussi and Gruss, 1996). The effect of the IL6RIL6 chimera can, therefore, be ascribed to a gene regulation effect on the Pax-3 homeobox gene, which is expressed during embryonic Schwann cell development prior to myelination, and has to be repressed for myelination to occur. Moreover, in degenerating demyelinating nerves, Pax-3 is re-expressed in the Schwann cells of when these cells stop to produce MBP. It is, therefore, of great importance that IL6RIL6 can both repress Pax-3 and cause the differentiation of Schwann cells into myelinating cells by inducing myelin protein genes.

Example 8: Injections of IL6RIL6 in a murine model of chronic relapsing multiple sclerosis.

Mice of the SJL/J strain develop experimental autoimmune encephalomyelitis (EAE) following immunisation with 0.4 mg bovine MBP in incomplete Freund's adjuvant containing 60 µg Mycobacterium tuberculosis H37Ra (Difco). The disease can be passively transferred to syngeneic recipient by intravenous injection of 30 million lymph node cells taken 10 days after immunisation. The clinical signs of paralysis appear after a week to 10 days. An acute phase of disease is followed later by remissions and relapses. IL6RIL6 is injected to these mice intraperitoneally or subcutaneously at doses of 1, 3 and 5 µg per mice (body weight about 25 g). The injections are given 4 times per week for at least 3 weeks, starting at either day 3 or day 7 after the passive transfer. The clinical score of the animals is followed and graded as: 1) loss of tail rigidity; 2) hindlimb weakness; 3) limb paralysis on one side; 4) limb paralysis on both sides; 5) lethality. The brain and spinal cord of animals is

examined by light microscopy following staining of myelin by luxol fast blue. The effect of IL6RIL6 on reduction in clinical grade and reduction of demyelination of white brain matter can be ascertained.

5 **Example 9: Neuroprotective effect of the IL6 chimera on neuronal and oligodendroglial neurocytotoxicity**

Organotypic cultures provide a unique strategy with which to examine many aspects of brain physiology and pathology. Long-term slice cultures from the hippocampus, a region involved in memory encoding and one that exhibits early degeneration in Alzheimer's disease
10 and ischemia, are particularly valuable in this regard due to their expression of synaptic plasticity mechanisms (e.g., long-term potentiation) and responsiveness to pathological insults (e.g., excitotoxicity).

The cultures were prepared as previously described by Bahr et al. (Bahr 1995) with minor modifications. Hippocampi were dissected out from eight day old Wistar rats and
15 400 µm thick slices were laid down on a porous and transparent Millicell-CM membrane (Millipore) and cultured in 6 wells multiwell plates. Four slices were placed on each membrane. Culture medium consisted of 50% minimal essential medium (+ 25 mM HEPES, + 4 mM NaHCO₃ + NaOH pH 7.2), 25% horse serum and 25 % Hanks' solution, glucose (6.4g/l), penicillin/streptomycin (10 ml/l), L-glutamine (2 mM). Cultures were kept in a 5%
20 CO₂ incubator set at a temperature of 37° C for at least 3 weeks before being used. Medium was changed every week.

Hippocampal slices were exposed to IL6 or to the IL6 chimera at concentrations ranging from 10 pg/ml to 10 µg/ml for 15 min, thereafter NMDA 50 µM was added for additional 30 min. The cultures were placed in fresh medium with or without IL6 or the IL6
25 chimera. At least four slices were used for each experimental point. NMDA-induced cell death was evaluated either 1 or 3-7 days after the experimental injury by incubating cultures for 1 hour in fresh medium containing propidium iodide (PI 5 µl/ml). Owing to its hydrophilic property, PI enters into disrupted cells and links to nuclear chromatin. The emitted red fluorescence (marker of cell death) was examined using an inverted fluorescence microscope

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(Zeiss) associated with an intensified charged-coupled device camera. Quantification of total fluorescence was performed by an image analysis system (Image Pro Plus).

Both IL-6 and the IL-6 chimera were found to protect from NMDA-induced hippocampal cell death. After one day in culture IL-6 at concentration ranging from 0.1 to 10 ng/ml, was found to protect approximately 50%-30% of the neuronal cells respectively, while the IL-6 chimera at concentration ranging from 0.05 to 1 pg/ml protected approximately 40%-75 % of the neuronal cells. The maximal protective effect of the IL-6 chimera (protection of 75% of the cells) was observed at a concentration of 0.5 pg/ml (Fig. 3).

The protective effects of IL-6 were found to decrease with time. Neuronal cell viability declined after treatment and after two days protective effect of IL-6 was maintained only with the 0.1ng/ml concentration of IL-6 with a protective effect on only 30 % of the cells at day 5. In contrast thereto, the IL-6 chimera at a concentration of 0.5pg/ml had a prolonged protective effect, maintaining approximately 60% of the cells for 5 days (Fig. 4).

The presence of oligodendrocytes in the organotypic slices was determined by immunohistological staining with Gal-C, a specific oligodendrocyte marker. After 2-3 weeks *in vitro* organotypic slices were exposed for 30 min. to 50 μ M NMDA, which induces necrotic cell death of the cells present in these cultures. In fact, as determined by RT/PCR, after treatment with NMDA all MBP expression that is distinctive to oligodendrocytes is lost in these cultures.

Activation of glutamate ionotropic receptors represents the primary event in the neurotoxicity process triggered by excitatory amino acids. Primary cultures of new born rat cerebella neurons (which contain >97% neurons) were employed for studying the effects of the IL6 chimera on glutamate-induced neurotoxicity and compared to the effects of IL-6.

Primary cultures of cerebellar granule cells were prepared from 8 day-old Sprague-Dawley rat pups as previously described (Pizzi et al. 1993). Cells were plated onto poly-L-Lysine-coated dishes and cultured in basal Eagle's medium containing 10% heat-inactivated foetal bovine serum, glutamine (2 mM), gentamicin (50 μ g/ml), and KCl (25 mM), at a density of 2.5×10^5 cells/cm². Cytosine Arabinoside (10 μ M) was added to the cultures 18 h after seeding to prevent non neuronal cell proliferation. Experiments were carried out after culturing the neurons for 10 days.

Unless otherwise indicated, cultures were exposed for 15 min to 50 μ M glutamate in a Mg^{2+} -free Locke's solution. IL6 or the IL6 chimera were added 5 min before glutamate treatment. Dishes were then returned to cultured conditioned medium at 37°C in 95% air / 5% CO₂ and cell viability was measured 18-24 hours later.

5 Cell viability was evaluated 18 h later by intravital staining with fluorescein diacetate and propidium iodide mixture, as previously described (Pizzi et al. 1993). The percentage of surviving neurons in the monolayer was computed by assessing the ratio between the fluorescein diacetate (green viable cells) and fluorescein diacetate plus propidium iodide staining (total cells) in photomicrographs of three representative fields from each monolayer.
10 Values were taken from three sister dishes.

While no protective effect on rat cerebellar granule cells was induced with IL-6 at doses ranging from 0.1 to 10 ng/ml, 0.1 pg/ml and 1pg/ml of the IL-6 chimera protected respectively 40-20% of the neuronal cells from glutamate-induced neurotoxicity.

15 **Example 10: Effect of IL6R/IL6 chimera on survival of superior cervical ganglia neurons after NGF withdrawal**

The neuroprotective effect exerted by the IL6R/IL6 chimera was also studied in a peripheral neuronal culturing system.

Superior cervical ganglia (SCG) neurons were isolated from P0-P3 (postnatal days 0
20 to 3) rat superior cervical ganglia. The neurons were kept for 4 days in a medium containing 5% rat serum and NGF. NGF is needed by the neuron for survival, whereas NGF deprivation leads to dying of the cells due to induction of apoptosis. The initial medium was replaced by NGF-free medium containing neutralizing antibodies to the growth factor. At this point of
25 time, 0.1 or 1 μ g/ml IL6R/IL6 chimera were added in 1% DMSO final concentration. The culture was kept at 37°C for 24 hours.

The effect of IL6R/IL6 chimera on the survival of NGF-deprived neurons after 24 hours of treatment was assessed by microscopy as well as by the mitochondrial activity of the cells as measured by the MTT assay, which is described in detail by Mosmann (1983). The concentrations of IL6R/IL6 chimera used did not show any toxic or morphological effect
30 on the SCG neurons treated with NGF.

Addition of IL6R/IL6 chimera in concentrations of 100ng/ml and 1 µg/ml rescued up to 40% of SCG neurons from neuronal death, which had been induced by NGF deprivation. as can be seen from the results of the MTT assay (Fig. 6). Thus, IL6R/IL6 chimera has a neuroprotective effect on peripheral neurons, suggesting an activity in neurodegenerative diseases like, e.g. Alzheimer Disease, Parkinson Disease or ALS (Amyotrophic Lateral Sclerosis).

Having now fully described the invention, it will be appreciated by those skilled in the art that the same can be performed within a wide range of equivalent parameters, concentrations and conditions without departing from the spirit and scope of the invention and without undue experimentation.



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CLAIMS

1. Use of IL6RIL6 chimera for the manufacture of a medicament for the treatment and/or prevention of neurological diseases and disorders.
- 5 2. The use according to claim 1, for manufacture of a medicament for treating traumatic nerve degeneration, demyelinating diseases of the CNS or PNS and/or neurodegenerative diseases.
- 10 3. The use according to claim 2, wherein the demyelinating disease is multiple sclerosis (MS).
4. The use of claim 2 wherein the neurodegenerative disease is selected from Alzheimer's disease, Parkinson's disease and ALS.
- 15 5. A pharmaceutical composition comprising IL6RIL6 chimera, optionally together with one or more pharmaceutically acceptable excipients, for treating and/or preventing of a neurological disease or disorder.
- 20 6. Pharmaceutical composition according to claim 5, comprising IL6RIL6 chimera, optionally together with one or more pharmaceutically acceptable excipients, for treating traumatic nerve degeneration, demyelinating diseases of the CNS or PNS and/or neurodegenerative diseases.
- 25 7. The pharmaceutical composition of claim 6 wherein the demyelinating disease is MS.

8. The pharmaceutical composition of claim 6 wherein the neurodegenerative disease is selected from Alzheimer's disease, Parkinson's disease and ALS.
9. A method for treating a neurological disease or disorder comprising administering to
5 a patient in need thereof an effective amount of IL6RIL6 chimera, optionally together with a pharmaceutically acceptable carrier.
10. A method for treating MS, Alzheimer's disease, Parkinson's disease or ALS comprising administering to a patient in need thereof an effective amount of
10 IL6RIL6 chimera, optionally together with a pharmaceutically acceptable carrier.

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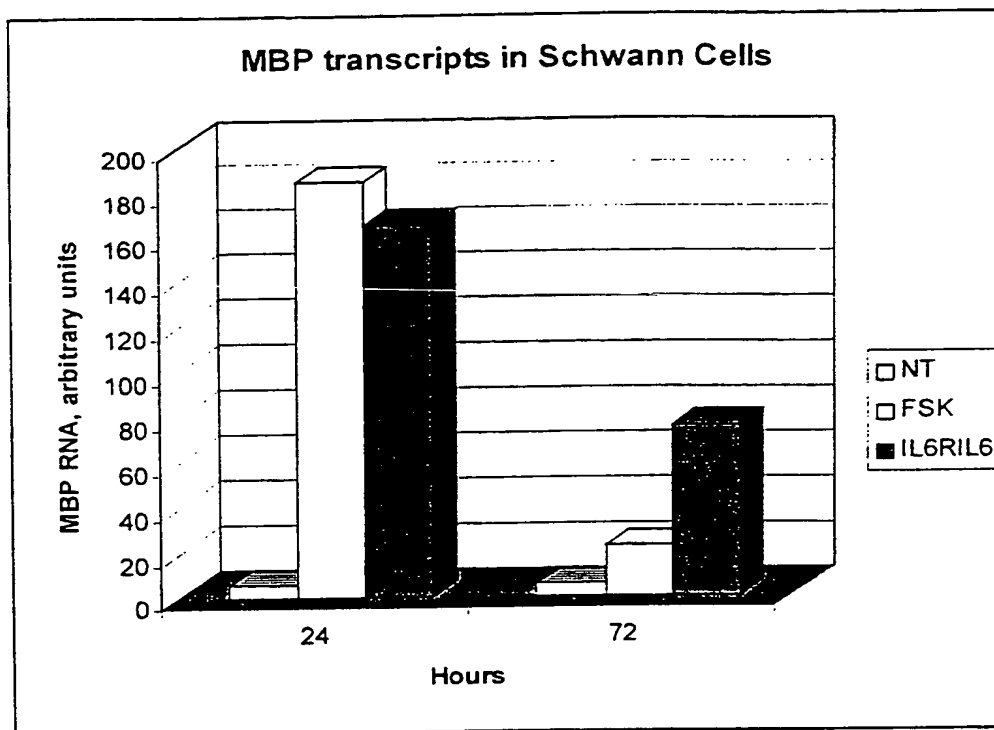


Fig. 1

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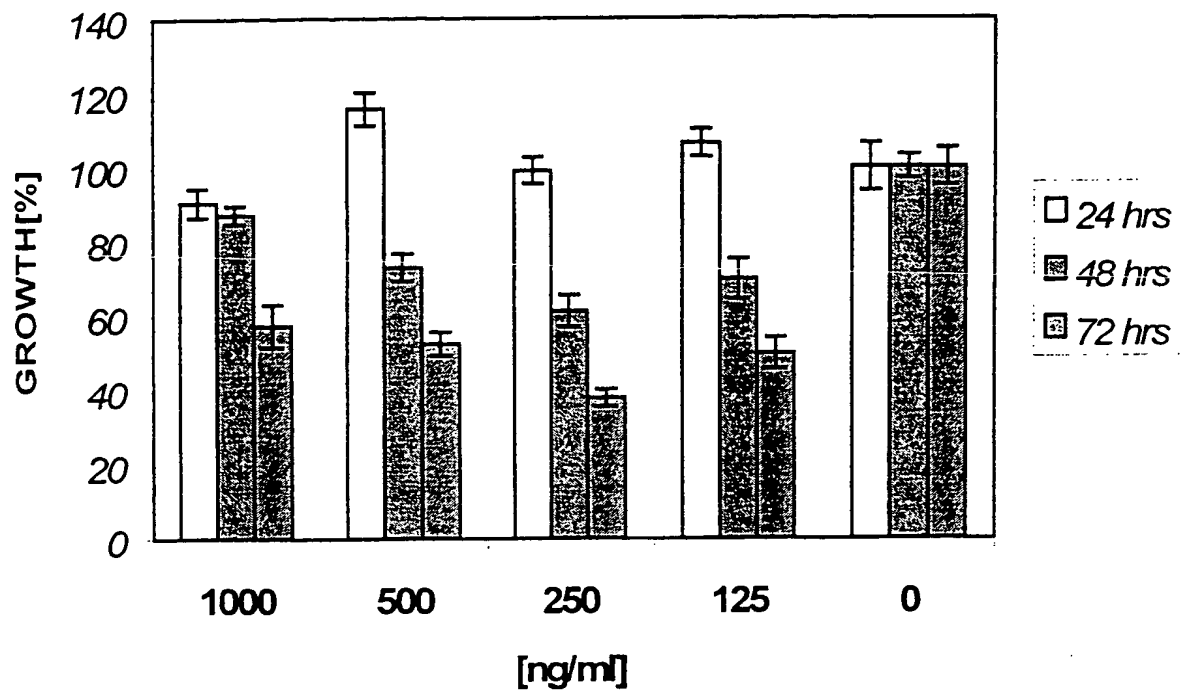
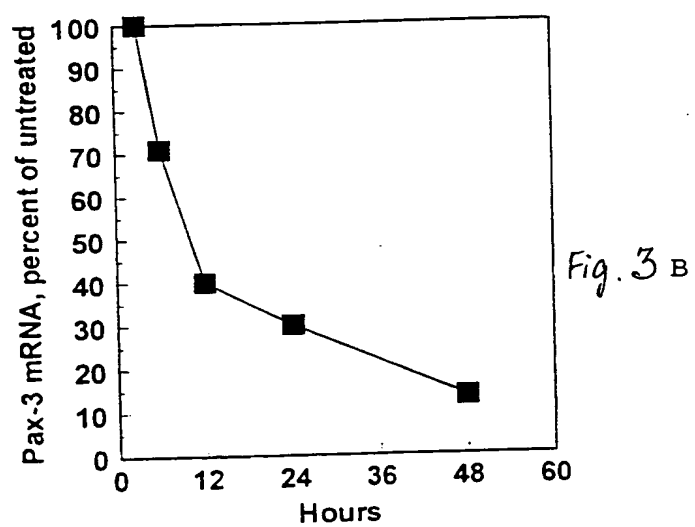
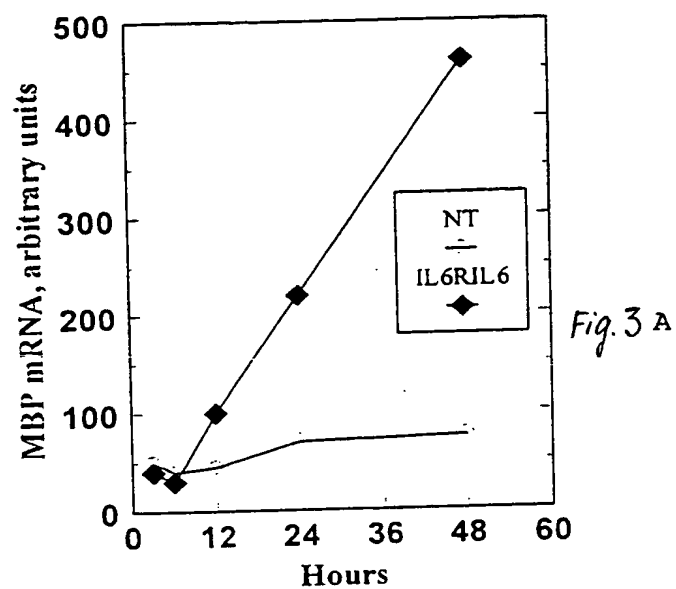


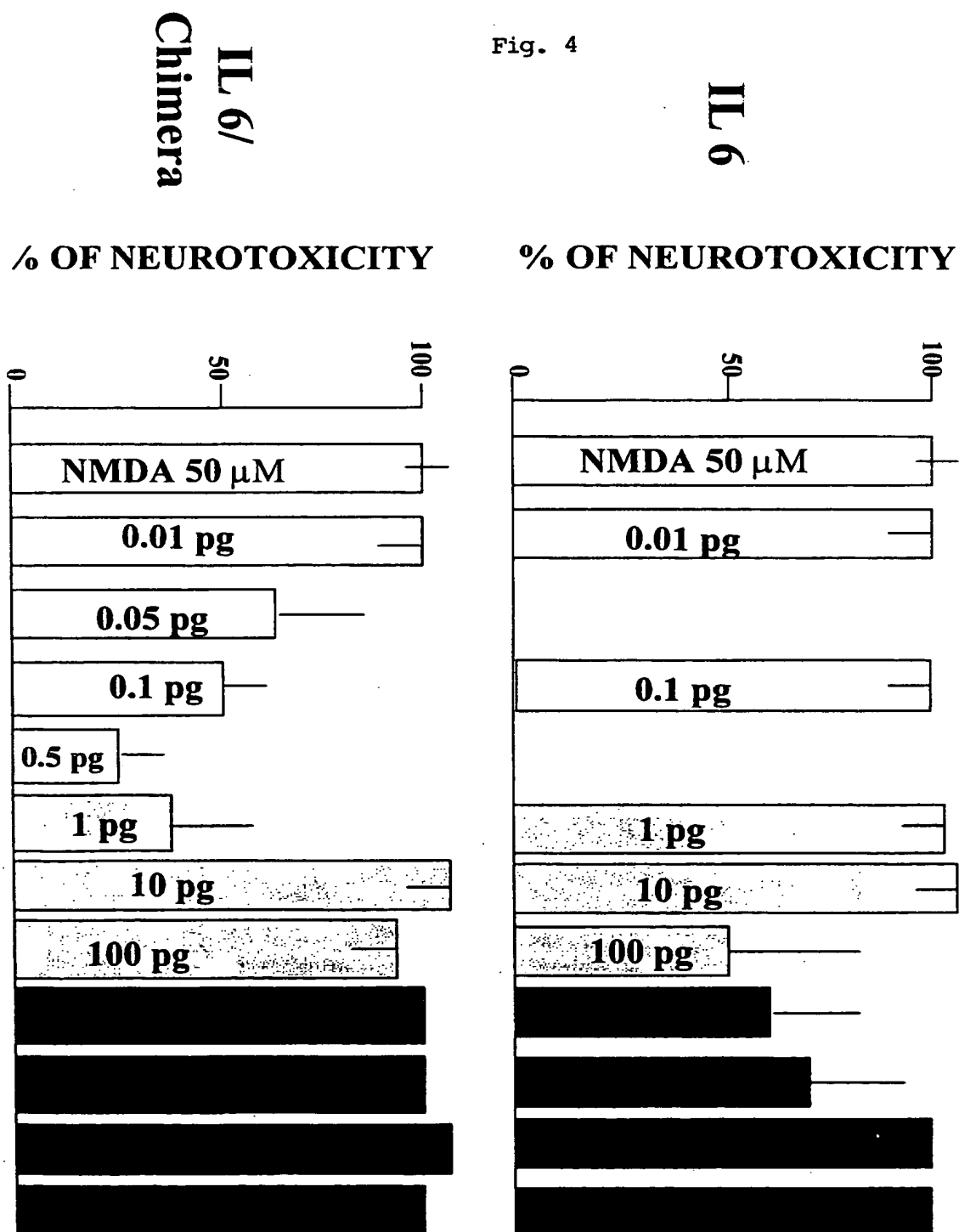
Fig. 2

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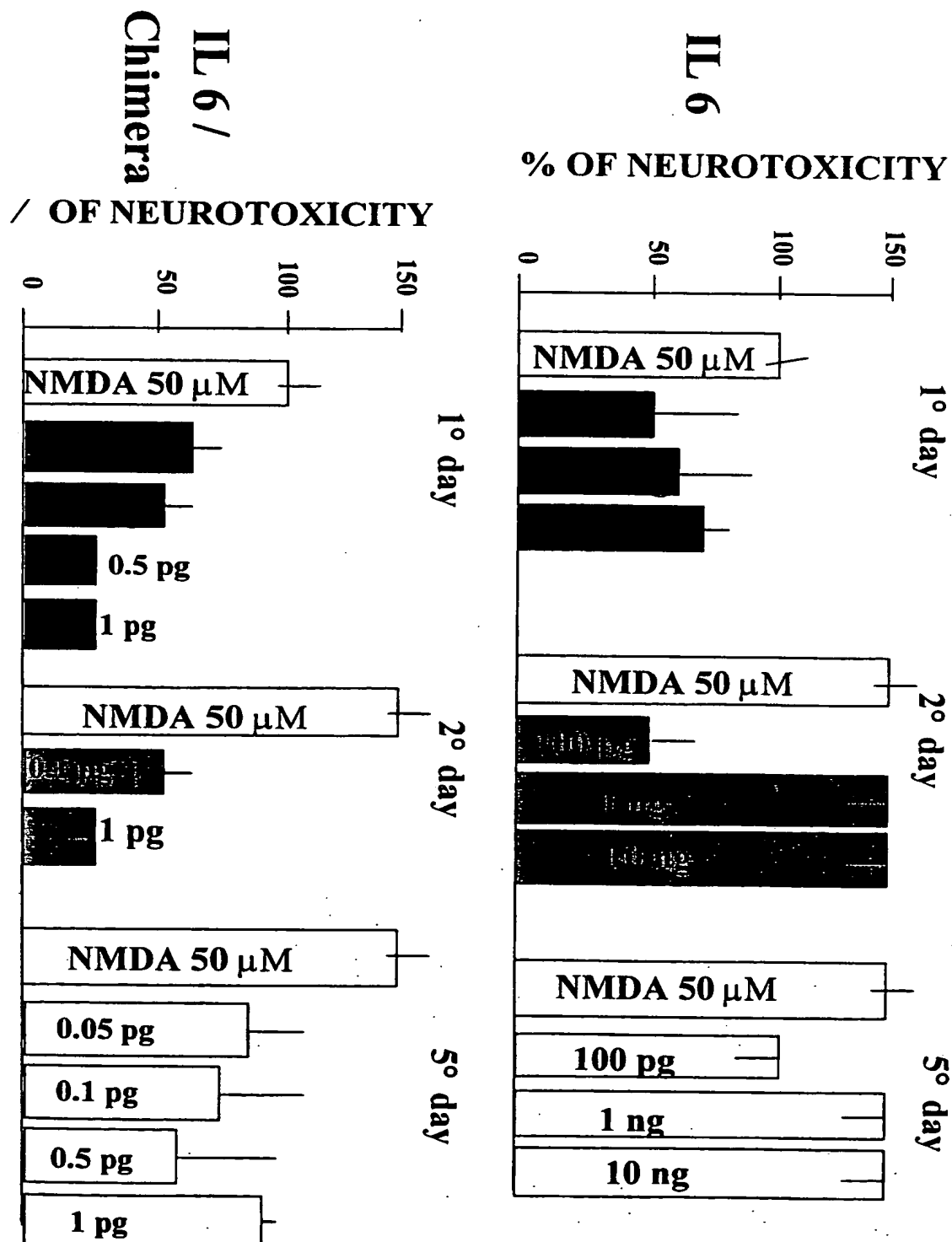
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Fig. 4



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Fig. 5



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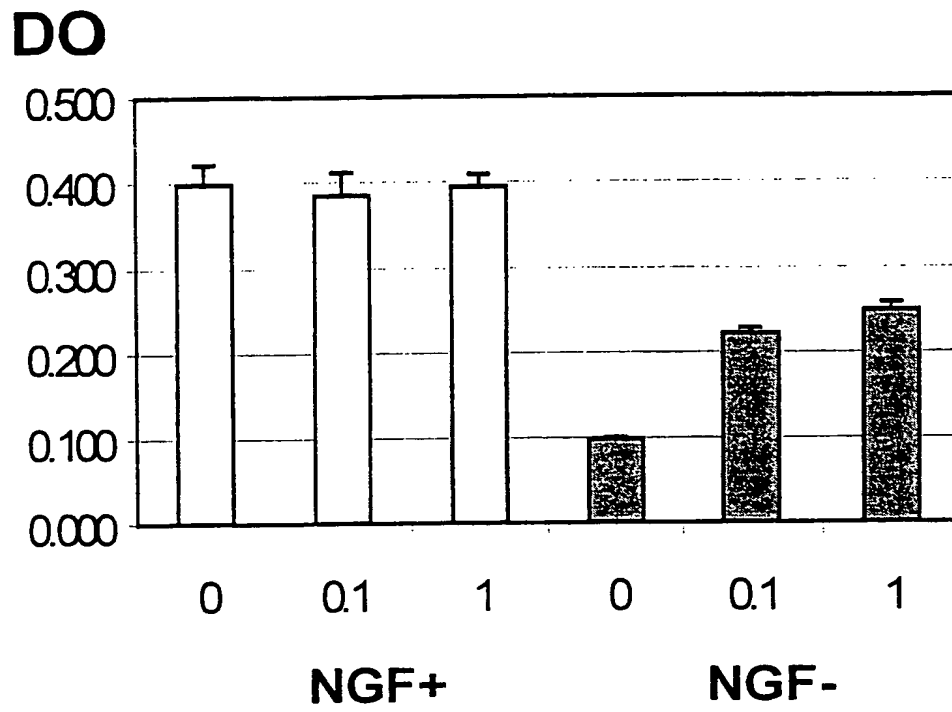


Fig. 6



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(54) Title: **IL6RIL6 CHIMERA FOR THE TREATMENT OF NEURODEGENERATIVE DISEASES**

(57) Abstract: The invention relates to the use of IL6RIL6 chimera in the treatment and prevention of neurological diseases and disorders.

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INTERNATIONAL SEARCH REPORT

Intern 1al Application No
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A. CLASSIFICATION OF SUBJECT MATTER
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According to International Patent Classification (IPC) or to both national classification and IPC

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Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

MEDLINE, CANCERLIT, AIDSLINE, LIFESCIENCES, CHEM ABS Data, SCISEARCH, BIOSIS, WPI Data, EPO-Internal, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 02552 A (KOLLET ORIT ; LAPIDOT TSVEE (IL); CHEBATH JUDITH (IL); REVEL MICHEL) 21 January 1999 (1999-01-21) cited in the application page 1, line 5-13 page 126-9 claims 1,31,32,36,37	1,5,9
A	RINGHEIM G E ET AL: "Enhancement of beta-amyloid precursor protein transcription and expression by the soluble interleukin - 6 receptor / interleukin - 6 complex." BRAIN RESEARCH. MOLECULAR BRAIN RESEARCH, (1998 MAR 30) 55 (1) 35-44. , XP000971298 the whole document	1-10

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

21 December 2000

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International Application No

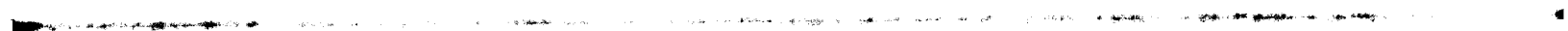
PCT/IL 00/00363

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International Application No

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